(19) Europäisches Patentamt
European Patent Office
Office européen des br v ts

(11) Publication number: **0 596 508 A1**

(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: **93117929.5**

(22) Date of filing: **04.11.93**

(51) Int. Cl.[5]: **C07K 15/00**, C12N 15/34, A01N 63/00

(30) Priority: **04.11.92 US 971624**

(43) Date of publication of application: **11.05.94 Bulletin 94/19**

(84) Designated Contracting States: **DE FR GB NL**

(71) Applicant: **BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.**
Tower Road
Cornell University
Ithaca, NY 14853(US)

(72) Inventor: **Granados, Robert R.**
5 Eagleshead Road
Ithaca, NY 14850(US)
Inventor: **Hashimoto, Yoshifumi, Dept.of Applied Biology**
Kyoto Inst.of Technology
Matsugasaki, Sakyo-ku 606(JP)

(74) Representative: **Buchner, Otto, Dr. et al**
Patentanwalt
Flossmannstrasse 30a
D-81245 München (DE)

(54) **Gene coding for a polypeptide which enhances virus infection of host insects.**

(57) Isolated and cloned baculovirus genes encoding a polypeptide protein present in the occlusion body of certain baculoviruses such as Trichoplusia ni granulosis virus and Pseudaletia unipuncta granulosis virus Hawaiian strain, said genes possessing a biological activity of enhancing virus infection of host insects by causing rapid degradation of the peritrophic membrane lining the midgut lumen of insects. The invention is also directed to pesticides incorporating said genes, i.e. the purified conserved and enhancing baculovirus proteins herein termed enhancins.

EP 0 596 508 A1

BACKGROUND OF THE INVENTION

The invention relates to the cloning and sequencing of novel viral genes from certain baculoviruses for insect control. More particularly, the invention relates to an isolated and cloned DNA from a granulosis virus which comprises an amino acid sequence of the viral gene encoding a polypeptide isolated from occlusion bodies of certain baculoviruses and which polypeptide possesses the biological activity of enhancing baculovirus infectivity. This invention also relates to isolated and purified baculovirus proteins which are characterized by enhancing the infectivity of baculoviruses. Such proteins termed herein as "enhancins" are found within the viral occlusion body, have a disruptive effect on the insect peritrophic membrane (PM) proteins, and/or interact with the midgut epithelium in such a manner as to permit the increased adsorption, penetration and uptake of virus particles by midgut cells with a concomitant increase in host mortality.

The publications used to illuminate the background of the invention, and in particular cases, to provide additional details respecting its practice are incorporated herein by reference, and for convenience, are numerically referenced by the following text and respectively grouped in the appended bibliography. Copies of all of the references mentioned in this bibliography are attached to the **INFORMATION DISCLOSURE STATEMENT** filed concurrently herewith.

FIELD OF THE INVENTION

Present in the protein occlusion bodies (OBs) of some baculoviruses is a unique viral-encoded protein which enhances viral infection of the host insect. This protein is referred to herein as the virus enhancing factor (VEF) and/or as the synergistic factor (SF). Pest control compositions comprising this factor and nuclear polyhedrosis viruses are the subject matter of U.S. Patent Nos. 4,973,667 and 5,011,685.

Studies on the mode of action of the VEF isolated from Trichoplusia ni (cabbage looper) granulosis virus (TnGV) showed that the VEF caused rapid degradation of the peritrophic membrane which lines the midgut lumen of lepidopterous larvae. Larval bioassays suggested that this alteration made the peritrophic membrane more permeable to invading baculoviruses resulting in at least a 25-fold increase in larval mortality (1,2).

DESCRIPTION OF RELATED ART

Closely related to, or identical with, the VEF protein is a lipoprotein, originally isolated in crude form from a Hawaiian strain of Pseudaletia unipuncta granulosis virus (PuGV-H), but not cloned or sequenced. It is described by Tanada and co-workers (7, 8, 10) as the "synergistic factor" (SF) and as having a calculated molecular weight between 90K and 160K (6, 24, 35, 42 and 43). The SF was released from the capsule upon dissolution in the midgut, and was the localized to the microvillar surface of the midgut cell membrane (9.37) where it caused an apparent increase in the uptake of enveloped nucleocapsids (36). The binding of SF to the midgut membrane was found to be specific with a calculated equilibrium constant of 1.57×10^{-9} M (39).

It has been postulated by Hashimoto et al (23) that the two proteins (VEF and SF) are closely related and have similar dual modes-of-action: peritrophic membrane disruption and increased virus uptake. Evidence to support this relationship comes from southern hybridizations of PuGV-H genomic DNA with the VEF gene and western blots of dissolved PuGV-H occlusion bodies with an anti-VEF polyclonal antiserum (23). Tanada determined that this SF in the capsule of PuGV-H increased the larval susceptibility to P. unipuncta nuclear polyhedrosis virus (PuNPV) (8).

Since viral enhancing proteins are important at early stages of host infection, it is important to identify and locate the position of the VEF gene and the SF gene on the viral genome. A need, therefore, exists to clone and sequence both the VEF gene of TnGV and the SF gene of PuGV-H. It is an object of this invention to satisfy such a need. Another object is to compare the SF and TnGV VEF genes by showing their extremely high degree of sequence similarity and by demonstrating their similar affects on T. ni PMs and AcMNPV infections in T. ni larvae. Still another object is to show sequence homology and/or serological relateness of the virulence genes and/or enhancing proteins among different baculoviruses.

SUMMARY OF THE INVENTION

The above-mentioned objects of the present invention, which will hereinafter become more readily apparent from the following description, have been attained by first isolating and purifying th VEF gene, which comprises a DNA molecule encoding a polypeptide of molecular weight 104 Kd and is found in the

granulin fraction of TnGV OBs purified by SEPHACRYL® S200 SUPERFINE (2.6 x 34 cm) column, possessing a biological activity and wherein said polypeptide has a total of 901 amino acid residues in the amino acid sequence of the polypeptide. Besides cloning and sequencing the gene encoding the viral enhancing factor (VEF) of TnGv, applicants have also successfully isolated the SF gene and determined its complete nucleotide sequence.

The gene encoding for the viral enhancing factor (VEF) of TnGv has been cloned from a lambda gtll expression library, and the complete nucleotide sequence determined. The VEF gene encodes a protein with a predicted molecular weight of 104 Kd which does not share homology to any previously reported proteins. The apparent promotor is located 4 bp upstream of the initiation codon and represents a consensus baculovirus late promoter (ATAAG). This has been confirmed by the identification of VEF mRNA in northern blots of infected larvae at 6 days but not 3 days post infection. Three repeats of the sequence 'TTACAAGA' which match the baculovirus late promoter in 4 of 5 nucleotide have been identified between 149 and 192 bp upstream of the initiation codon. While the function of these sequences is unknown, they are not believed to be transcriptionally active since they diverge from the consensus promoter at the invariant 'T' position. Using the VEF gene as a probe in southern blots of genomic DNAs, homologous sequences have been identified in PuGV-H and Heliothis armigera GV (HaGV) but not Erinnyis ello GV, (EeGV), Autographa californica nuclear polyhedrosis virus (AcMNPV) or Trichoplusia ni nuclear polyhedrosis virus (TnSNPV). In addition, SDS-PAGE analysis of dissolved viral occlusion bodies have demonstrated proteins with a molecular weight similar to VEF in PuGV-H and HaGV.

As pointed out above, the gene encoding the synergistic factor (SF) of PuGV-H has been cloned by applicants and the complete nucleotide sequence determined. The SF gene encodes a protein with a predicted molecular weight of 104Kd which shares a 99.1% and 98.2% homology with the nucleotide and amino acid sequence of the viral enhancing factor (VEF) gene of TnGv, respectively. A majority of the differences in the amino acid sequences of the two viruses result from two reciprocal frameshifts which occur between nucleotide +1962 and +1985 of the SF gene. Both enhancing proteins have similar activity in neonate larvae of T. ni (2.4 fold enhancement) and **in vitro** peritrophic membrane assays. Using a polyclonal antibody directed against TnGV VEF, 17 baculoviruses were screened by western blot hybridization. Cross reactive proteins are found in seven GVs isolated from 4 families of Lepidoptera. These putative enhancing proteins can be separated into 3 groups based on size: HaGV (110Kd); PuGV-H, Pieris rapae GV (PrGV), **Scotogramma trifolii** GV (StGV), and TnGV (104Kd); and **Cydia pomonella** GV (CpGV) and **Estigmene acrea** GV (80Kd). The name "enhancin" has been proposed for these enhancing proteins.

DESCRIPTION OF THE DRAWINGS

A more complete appreciation of the invention and the attendant advantages thereof will be readily attained as the same becomes better understood by reference to the following details of description when considered in connection with the accompanying drawings, i.e. Figures 1-11.

Fig. 1. Mapping of the VEF gene of TnGV. a) a Hind III restriction map of the TnGV genome. By convention, the smallest fragment containing all of the granulin gene is assigned to be the first fragment at the left of the linearized map. A fine map of the b) Hind III-M fragment of TnGV and c) fusion gene of lambda F. The striped box indicates the position of the VEF gene while the open box indicates non-coding TnGV sequences inserted into the lambda gtll. The entire insert in lambda F is demarcated by the asterisks. The size of the DNA is indicated by scale, and the restriction sites for BamHI(B), ClaI(C), EcoRI(E), HindIII-(H), KpnI(K), and SalI(S) are indicated.

Fig. 2. Western bolt analysis of lambda lysogens from lambda F (lane 1 or lambda gtll (lane 2) probed with either anti-VEF polyclonal antibody (lane 1) or an anti-β-galactosidase monoclonal antibody (lane 2). Lysogens were first separated on a 10% SDS-PAGE gel and then electrophoretically transferred to nitrocellulose. The 153Kd protein identified by the anti-VEF polyclonal antibody consists of 39Kd VEF carboxy-terminal and 114Kd of β-galactosidase.

Fig. 3. The nucleotide sequence of the VEF gene from TnGV. The gene has been translated using the single-letter amino acid code. The bolded sequence represents the consensus baculovirus late promoter (5), and the underlined sequences represents 3 repeats of the sequence (TTACAAGA) which matches the promoter in 4 of the 5 base pairs. Double underlined sequences indicate possible glycosylation sites. The DNA sequence of a 3.5 Kb portion of Hind III-M fragment was determined by dideoxy chain termination method using bacteriophage T7 DNA polymerase. Sequence data were compiled and analyzed using the software program of PCGENE. In this sequence, A stands for deoxyadenyl, G for deoxyguanyl, C deoxycytidyl, and T is thymidyl. The amino acids encoded by the above DNA are designated below the appropriate nucleotide triplet. Accordingly, M is methionine; K is lysine, P is proline; E is glutamate, L is

leucine; T is threonine; A is alanine; S is serine; V is valine; F is phenylalanine; I is isoleucine; G is glycine; D is aspartic acid; Q is glutamine; R is arginine; C is cysteine; W is tryptophan; N is asparagine; H is histidine; and Y is tyrosine.

Fig. 4. Northern blot of total RNA isolated from infected larvae. Total RNA was isolated from T. ni larvae at 3 and 6 days post inoculation (PI) with TnGV. Ten micrograms of RNA were electrophoresed in a denaturing 1.5% agarose and northern blotted following the methods of Dwyer and Granados (17). Blots were probed with the internal KpnI fragment of TnGV-VEF gene under high stringency conditions. No hybridization was found to RNA isolated at 3 days PI. However, 2 RNA species of 2.7 and 3.3 Kbp hybridized at 6 days PI. This indicated that the VEF gene was probably a late gene.

Fig. 5. Southern hybridization and SDS-PAGE analysis of TnGV and 5 other baculoviruses. a) Genomic baculovirus was digested with Hind III and electrophoresed on a 0.75% agarose gel. The DNA was transferred to nitrocellulose and probed with the internal KpNI fragment of TnGV-VEF and washed under high stringency conditions. Homologous sequences were identified in PuGV-H, and HaGV. b) Occlusion bodies are dissolved in 0.05 M NaCO pH 10.5 for 15 minutes at room temperature and the nucleocapsids pelleted by centrifugation at 14,000 xg. The supernatants were removed and electrophoresed in a 10% SDS-PAGE gel and stained with COOMASSIE blue.

Fig. 6. A comparison of the nucleotide sequence for the PuGV-H SF and TnGV VEF genes. Hyphens denote nucleotide identical to the PuGV-H sequence. The consensus baculovirus late promoters (30) have been underscored, the putative start codon has been bolded, and the stop codon overscored. Two frameshift mutations have occurred at +1962 and +1985. The homology between the two genes is 99.1%. The second open reading frame starts at +2755 nt. The stop codon for this gene has not been found.

Fig. 7. A comparison of the amino acid sequence for the PuGV-H SF and TnGV VEF proteins. Hyphens denote nucleotide identical to the PuGV-H sequence. The identity between the two proteins is 98.2%.

Fig. 8. SDS-PAGE analysis of purified SF and VEF. Three micrograms of VEF or SF was added to an equal volume of 2X SDS-PAGE loading buffer (0.125 M Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol). The sample were electrophoresed for 4.5 hours at 30 mAmps through a 7% separating gel (10 cm x 12 cm x 1.5 mm), and stained with COOMASSIE blue R-250 following standard protocols. SF (lane 1) migrated at 106K while VEF (lane 2) migrated at 101K.

Fig. 9. SDS-PAGE analysis of in vitro digest of peritrophic membranes. Individual peritrophic membranes (PM) were dissected from the last instar (a) **Trichoplusia ni** and (b) **Pseudaletia unipuncta** larvae. Peritrophic membranes were resuspended in 50 µl of 0.1 M Na_2CO_3 pH10.5 containing (a) 5 µg or (b) 10 µg of VEF (lane 2) or SF (lane 3), and incubated at 28° C for 60 min. The reactions were stopped by washing the PMs in deionized water and resuspending in SDS-PAGE loading buffer (0.062 M Tris-HCl pH 6.8, 2% SDS, 5% - mercaptoethanol). Controls (ln 1) consisted of PMs treated in the same manner but without enhancing protein. Samples were electrophoresed through at 10% separating gel and silver stained. SF and VEF digested the same protein in both the **T. ni** and **P. unipuncta PMs**. Multiple degradation products are evident in both the SF (ln 3) and VEF (ln 2) lanes.

Fig. 10. SDS-PAGE and Western bolt analysis of nine baculoviruses. Viral occlusion bodies were dissolved in DAS (0.1 M Na_2CO_3, 0.01 M EDTA, 0.17 M NaCl, pH 10.9) for 15 min at room temperature. An equal volume of 2X SDS-PAGE loading buffer (0.125 M Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol was added and the samples electrophoresed through a 10% separating gel. The gels were either (a) stained with COOMASSIE blue (b) or used in Western blots. Proteins cross-reacting with an anti-VEF/TrpE (23) antiserum were found in CpGV (lane 1, 80Kd), EaGV (Lane 2, 80Kd), HaGV (lane 4, 110Kd), PrGV (lane 7, 101Kd) and PuGV-H (lane 8, 106Kd). EeGV (lane 3), PbGV (lane 5), PiGV (lane 6), PuGV-O (lane 9) did not cross-react with the antiserum.

Fig. 11. SDS-PAGE and Western blot analysis of nine baculoviruses. Viral occlusion bodies were dissolved in DAS (0.1 M Na_2CO_3, 0.01 M EDTA, 0.17 M NaCl, pH 10.9) for 15 min at room temperature. An equal volume of 2X SDS-PAGE loading buffer (0.125 M Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol) was added and the samples electrophoresed through a 10% separating gel. The gels were either (a) stained with COOMASSIE blue (b) or used in Western blots. Proteins cross-reacting with an anti-VEF/TrpE (23) antiserum were found in StGV (lane 11, 106Kd), and TnGV (lane 12, 104Kd). SfGV (lane 10), AcMNPV (lane 13), AgMNPV (lane 14), CfNPV (lane 15), HzSNPV-ELCAR (lane 16), and TnSNPV (lane 17), did not cross-react with the antiserum.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Enhancing Protein Purification

The TnGV VEF and PuGV-H SF were isolated according to the methods of Gallo et al. (21) with the following modifications. Sephacryl S-200 was replaced by Sephacryl S-300 HR and the initial concentration of viral occlusion bodies was reduced from 1.7×10^{12} to 1.0×10^{12} per ml.

Purified enhancing factor containing approximately 3 mg of protein was added to an equal volume of 2X sample buffer (2X = 0.125 M Tris-HCl pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol), heated in a boiling water bath for 3 min., and separated by SDS polyacrylamide gel electrophoresis (PAGE) according to the methods of Laemmli (29). Electrophoresis was carried out at 30 m Amps for 4.5 hr in a 7% separating gel (10 cm x 12 cm x 1.5 mm), and stained with COOMASSIE Blue R-250 following standard protocols.

Cloning and Sequencing of Enhancing Genes

The VEF present in granulin fraction of TnGV OBs was purified in the following manner:

1.7×10^{12} TnGV OBs were dissolved in 1 ml 0.05 M Na_2CO_3 for 15 min. at room temperature, and layered on a 20% sucrose cushion in H_2O and centrifuged for 45 min. at 126,000 g at 4 °C. The granulin fraction remained on top of the sucrose cushion and was collected. After an incubation of 5 hrs at 28 °C, the granulin fraction was applied onto a Sephacryl-S-200 column (2.6 x 34 cm) and eluted with 50 mM Tris-HCl pH 7.0, 0.1 M NaCl at 1.5 ml/min, and the absorption of the eluate measured at 280 nm. The first peak containing VEF protein was poled and used for experiment.

A cloning and expression vector, lambda gtII, was used for construction of genomic library of TnGV and for isolation of the VEF gene (3). Antibodies were raised against Sephacryl-column purified VEF from granulin fraction after alkali solubilization of OBs (1) and were used for immunoblotting to screen for positive clones. Through several steps of screening approximately 6000 plagues, a clone was selected containing the longest viral-VEF DNA insert. Southern blot hybridization analysis of TnGV DNA Hind III digests, probed with the VEF clone insert, revealed that the VEF gene existed on the Hind III-M fragment. Western blot analysis of the fusion protein expressed in lysogenic E. coli (Y1089 strain) transfected with VEF clone had a molecular weight of 153 kD (Fig. 2). This suggested a fusion protein gene consisting of 39 Kd of the VEF carboxy terminal end and the 114 Kd beta-gal gene (Fig. 1c, 2). Since the VEF has a size of 104 Kd, the position of the VEF gene on a fine map of the Hind III-M fragment was predicted and a 3.5 kbp DNA portion was sequenced (Fig. 1b).

Sequence analysis showed an open reading frame of 2,703 bp DNA corresponding to the size of the VEF polypeptide at the predicted location of the VEF gene (Fig. 3). The deduced size of the polypeptide was 104,300 daltons and consisted of 901 amino acid residues. There are no sites for lipophilic modification (Lys/X/X/Cys/X/X/Asn). To determine the presence of the VEF gene among several isolates of baculovirus a 1.5 kbp portion of the VEF gene was probed onto a Southern blot of different virus DNA fragments digested with Hind III restriction enzyme under high stringency condition (12). The result showed that two granulosis virus DNAs, isolated from PuGV-H and HaGV, contained a sequence homologous to the TnGV VEF gene probe (Fig. 5a). DNA isolated from EeGV did not contain sequences homologous to the VEF gene probe. The restriction enzyme digestion pattern of DNA from TnGV, GVH, and HaGV were very similar, whereas EeGV exhibited a very distinct DNA Profile. The probe did not hydridize with DNAs from two nuclear polyhedrosis viruses (Fig. 5a). Temporal gene expression of the VEF gene was examined by Northern blot analysis of total RNA from TnGV-infected T. ni larvae at 3 and 6 days p.i. A probe with a size of 1.5 Kb KpnI-V fragment, a part of the VEF gene, showed no hybridization with RNAs at 3 days p.i. but showed strong hybridization with two RNA species with sizes of 2.7 Kb and 3.3 Kb at 6 days p.i. (Fig. 4). The TnGV-VEF present in the granulin fraction of alkaline dissolved OBs was resolved as a 104 Kd protein on a SDS-polyacrylamide gel. To determine the presence of high molecular weight polypeptides in granulin or polyhedrin fractions from six baculoviruses, these virus samples were analyzed by SDS-PAGE (Fig. 5b). In the granulin fractions from TnGV, GVH and HaGV, polypeptides with a size of 104 Kd, 106 Kd, and a complex of 110 Kd and 94 Kd were detected, respectively. The single high molecular weight polypeptide (106 Kd) from GVH appears to migrate on SDS-PAGE similar to the 104 Kd protein from TnGV (Fig. 5b and Y. Tanada, personal communication). The assignment of a VEF function to either the 94 or 110 kDa polypeptides from HaGV is not clear at this time. No polypeptides with a size of approximately 100 kDa were present in EeGV, TnSNPV, and AcMNPV. Three of the GVs examined, TnGV, GVH, and HaGV all infect the noctuid sp cies T. ni, whereas EeGV grows only in the sphyngid species, E. ello.

KpnI and SalI subclones of the EcoRI-I fragment of PuGV-H, which contains the entire SF gene, were cloned into the KpnI and SalI sites of pUC 19 (40). This was followed by nested deletions from both ends of the subcloned DNA using the Exo/mung deletion kit (Stratagene). The nucleotide sequence was determined using the dideoxy chain termination method of Sanger et al. (32) as modified for use with the Sequenase sequencing kit (U.S. Biochemicals). Sequencing data were compiled and analyzed using the PCGENE (Intelligenetics) software package.

Neonate bioassays employing 3-5 hr old larvae were conducted according to the methods of Hughes et al. (28) except that the neonates were not preselected for vigor and the droplets were applied by means of a syringe equipped with a blunt needle (26). The inoculum contained 1 x 10^5 OB/ml of AcMNPV with 1.0 or 0.5 mg/ml of either TnGV VEF or PuGV-H SF and all larvae were assumed to have imbibed 10 nl of inoculum (27). After ingestion of the inoculum, larvae were transferred with a fine paintbrush into individual 35 ml cups containing high wheat germ diet. Controls consisted of neonates that imbibed either virus without any enhancing factor or water with food coloring. The test was conducted 2 times with 30 larvae/treatment in each test group.

In Vitro peritrophic membrane assay

PM were dissected from last instar T. ni. and P. unipuncta larvae, rinsed in deionized water to remove diet residue, and stored at -80 • C. Thawed PMs were resuspended in 50 ml of digestion buffer (o.1 M Na_2CO_3, pH 10.5) containing either 5 µg or 10 µg of SF or VEF. After incubation at 28 • C for 1 hr, the PMs were washed in water, placed in 1X SDS-PAGE sample buffer and boiled for 5 min. Controls consisted of PMs treated in the same manner but without any enhancing factor. The protein composition of the treated and control PMs were analyzed by discontinuous SDS-PAGE (79) on a Mini-PROTEAN II (BioRad) at 200 volts for 35 min with a 10% separating gel. Gels were stained using the BioRad Silver Staining Kit according to he manufacturer's instructions.

Western blots.

Viral occlusion bodies were first dissolved in dilute alkali solution (0.1 M Na_2CO_3, 0.01 M EDTA, 0.17 M NaCl, pH 10.9) for 15 minutes at room temperature. An equal volume of 2X SDS-PAGE sample buffer was added and the mixture heated in a boiling water bath for 7 minutes. Samples were separated by SDS-PAGE as described above. The proteins were then electrophoretically transferred to nitrocellulose paper following the methods of Towbin et al (38). Western blots were analyzed using an anti-VEF/TrpE polyclonal antibody at a dilution of 1:5000 (23). Bands were visualized using an alkaline phosphatase conjugated secondary antibody (19).

SUMMARY OF RESULTS

For cloning and sequence analysis of VEF, two positive clones were identified from the approximately 6000 plaques screened with a α-VEF polyclonal antiserum. Both clones had identical inserts of 2.8 Kb mapped to the Hind III-M fragment of the TnGV genome (92.2 to 95.8 map units; Fig. 1a). Other TnGV fragments hybridizing to the clones included the 6.7 Kb EcoRI-K and the BamHI-FG doublet. Detailed maps of both TnGV Hind III-M and the insert DNA were generated using several restriction enzymes (Fig. 1b,c).

Western blot analysis using both an anti-VEF polyclonal antisera and an anti-β-galactosidase monoclonal antibody (Promega, Madison, WI) demonstrated that the fusion protein generated by lambda-F had a molecular weight of 153 Kd which presumably consisted of 39 Kd of VEF carboxy-terminal and 114 Kd of β-galactosidase protein (Fig. 2). The VEF gene was tentatively positioned on the Hind III-M fragment using this information.

Sequence analysis of approximately 3.5 Kbp of Hind III-M DNA revealed an open reading frame of 2703 bp (901 amino acids) encoding a protein with a predicted molecular weight of 104.3 Kda (Fig. 3). The predicted protein contains 12 candidate sites for N-linked glycosylation (ASN/X/SER or Thr) and no sites predicted for lipophilic modification (LYS/X/X/CYS/X/X/ASN). A consensus baculovirus late promoter (ATAAG) occurred at -4 nt and a probable polyadenylation signal (AATAA) was found 2 nt downstream of the VEF ORF. The upstream region of the VEF gene contained three perfect repeats of the sequence TTACAAGA between -192 and -149 nt of the translation start site. Curiously, these repeats were similar to the baculovirus consensus sequence for hyperexpression described by Rohrman (5). However, in all three sequences, mismatches occurred at the invariable "T" of the "ATAAG" core late promoter motif. Changes at this position have been shown to eliminate transcriptional initiation (5). A comparison of the deduced

amino acid sequence of the VEF with both the NBRF and Swiss-Prot protein data bases did not reveal any similarity to known proteins.

The occurrence of the late core promoter sequence at -4 bp indicated that VEF should be expressed late in infections. This was demonstrated by isolating RNA from infected larvae at several times (3 days and 6 days) PI. Using a restriction fragment from within the VEF open reading frame (ORF) as a probe, strong hybridization was shown to 2 RNA species (2.7 and 3.3 Kb) at 6 days PI but none at 3 days PI (Fig. 4). The transcript size of 2.7 Kb agreed with the predicted transcription start and stop signals adjacent to the open reading frame.

For SF sequence analysis, approximately 3300 bp within the EcoRI-I fragment of PuGV-H was sequenced, in both directions, revealing a 2703 bp open reading frame (ORF; Fig. 6) with a calculated molecular weight of the protein of 104Kd. A consensus baculovirus late promoter motif (ATAAG; Ooi et al.) (30) was located at -8 to -4 nts relative to the ORF. A comparison of both the nucleotide and amino acid sequence with that of the VEF gene from TnGV revealed a 99.1% (Fig. 6) and 98.2% (Fig. 7) homology respectively. The only significant difference in homology between PuGV-H SF and TnGV VEF genes occurs between nucleotide +1962 and +1985. Two reciprocal frameshifts in the PuGV-H sequence have caused a 7 amino acid gap which shares no homology to the TnGV VEF protein sequence. Homology of the PuGV-H gene with the TnGV VEF gene was greater than 95% for 300 bp upstream of the gene. After this point, the homology decreases to 17.7%. From data analyzed thus far, the PuGV-H and TnGV sequence homology is greater than 99% for 155 nts downstream of the genes. A consensus baculovirus late promoter motif is located 35 nt upstream of the stop codon of the VEF and SF gene sequences, and 78 nt upstream of a potential ORF. This possible second ORF is located 43 nt downstream of the SF and VEF ORFs.

The enhancing protein from PuGV-H was purified from capsules in the same manner as the TnGV VEF. Approximately 330 mg of purified protein was isolated from 1.0×10^{12} OBs. Based on SDS-PAGE analysis, purified SF had a calculated molecular weight of 106Kd (Fig. 8). While this was in good agreement with the predicted molecular weight of 104Kd from other protein sequence analysis there is a repeatable difference in the migration pattern when compared to the TnGV VEF protein (Fig. 8).

The homology between the VEF and SF proteins suggested that the ability to enhance baculovirus infections should also be similar. This was tested by a neonate larval bioassay (Table 1) and an in vitro PM assay (Fig. 9). Enhancement of AcMNPV infections of T. ni larvae occurred with the VEF and SF proteins. The 2.4 fold enhancement of

TABLE 1

Effect of PuGV-H and TnGV Enhancing Factors on AcMNPV Infections of *Trichoplusia ni* Neonate Larvae*

Enhancing Factor		Percent Mortality†
Source	ng/larva	
PuGV-H	10	95
PuGV-H	5	95
TnGV	10	95
	0	40

* All larvae were infected with 1 OB.

† This represents the average of two bioassays with 30 larvae per treatment. Non-virus control had no mortality.

infections by SF was identical to that seen in the VEF assays. In the in vitro PM assay, SF and VEF digested the same proteins in both the T. ni and P. unipuncta PMs. For the T. ni PM, 3 proteins of molecular weight 36.6Kd, 111.5Kd, and 98.2Kd present in the control lanes are absent in the SF and VEF treatment lanes (Fig. 9a). Protein bands found only in the treatment lanes include a predominant group of bands occurring between 71.6K and 58.8K and 2 lower molecular weight proteins of 31.2K and 23.3K (Fig. 9a). In P. unipuncta, 7 proteins are absent in the treatment lanes as compared to the control (Fig. 9b, lane 1). The molecular weight of the digested bands are 210.5Kd, 184.3Kd, 171.1Kd, 125.7Kd, 111.5Kd, 36.4Kd, and 32.0Kd. While there are 4 new protein bands of molecular weight 182.4Kd, 121.3Kd, 32.4Kd, and 24.6Kd common to both SF and VEF treatments, 4 unique proteins are also evident: 85.5K in VEF and 91.8K, 82.7K, and 80.0K in the SF treatment (Fig. 9b, lanes 2 and 3).

In order to ascertain the prevalence of the VEF gene within the Baculoviruses, 17 different baculoviruses (12 GVs and 5 NPVs) have been screened for VEF homologs using a polyclonal antisera specific for the TnGV VEF protein (Fig. 10 and 11). Cross-reactive proteins were found in 7 GVs: Cydia pomonella GV (CgGV), Estigmene acrea GV (EaGV), HaGV, PrGV, PUGV, and StGV. This represents GVs which were isolated from 4 different families of Lepidoptera: Arctiidae, Noctuidae, Pieridae, and Tortricidae. EeGV, PuGV Oregon strain, Plodia interpunctella GV, Pieris brassicae GV, and Spodoptera frugiperda GV did not have any cross-reactive proteins. None of the NPVs (AcMNPV, Anticarsia gemmatalis MNPV, Choristoneura fumiferana NPV, Helicoverpa zea SNPV, and TnSNPV) reacted with the antisera.

The identified VEF cross-reactive proteins could be subdivided based on the molecular weight of the proteins. PrGV, PuGV-H, StGV, and TnGV had the most common protein size of approximately 104Kd. HaGV had a slightly higher molecular weight (110Kd) while CpGV and EaGV had a significantly lower molecular weight of approximately 80Kd.

The cloning and sequencing of the SF gene from PuGV-H represents the second baculovirus enhancing factor to be sequenced to date. The high degree of homology between the PuGV-H and TnGV genes is unusual and indicates that there may be a strong selective pressure on the gene. Another possible explanation is that PuGV-H may be a variant of TnGV: however, this seems unlikely since the degree of homology decreases to 17.6% 300 bps upstream of the gene. In addition, there are significant differences in the restriction enzyme patterns of the two viral genomes (23). The identification of a second open reading frame may explain the high degree of homology (> 99%) observed downstream of the two genes. The effect of this downstream gene on the expression of VEF or SF is unknown. On SDS-PAGE, the SF and VEF proteins show a consistent difference in mobility. Since these proteins have near identical molecular weights it is possible that the two proteins may be processed or modified differently in the two hosts.

The results from both the neonate and in vitro PM assays demonstrate that the two proteins are very similar in activity. The observed differences in the digestion patterns of the PM proteins from both T. ni and P. unipuncta are probably due to quantitative differences in the amounts of the two enzymes. Evidence for this comes from the T. ni digests and the periodicity of the protein bands between 71.6Kd and 58.8Kd. The same bands are present in both the SF and VEF digests; however, the intensity of the bands differ. In the VEF digest (Fig. 9a, lane 2) the higher molecular weight bands predominate while in the SF digest (Fig. 9a), lane 3) the opposite is true. The data suggests a possible endoproteolytic type of cleavage in which the digestion in the SF reaction has proceeded further than in the VEF reaction.

Five baculoviruses were originally tested for the presence of VEF-homologous proteins by both DNA hybridization and SDS-PAGE analysis of dissolved occlusion bodies (Fig. 4A and B) Hind III genomic digest of the 5 baculovirus DNAs under low stringency conditions, using a restriction fragment with the VEF ORF as a probe, showed homology between TnGV and 2 other granulosis viruses (PuGV-H and HaGV). No apparent homology was seen to either EeGV, TnSNPV, or AcMNPV (Fig. 5A).

To date, a total of 8 GVs have been reported to have enhancing proteins. Seven of the proteins cross-react with a polyclonal antiserum specific for the VEF from TnGV. The other enhancing factor, which is found in Xestia c-nigrum (23), has not been tested with the antiserum. Enhancing proteins have now been identified in baculoviruses isolated from four families of Lepidoptera: Arctiidae, Noctuidae, Pieridae, and Tortricidae. Previously, enhancing factors had only been identified in GVs infecting Noctuidae. This data lends credibility to the hypothesis that these enhancing proteins are common in GVs and are important baculovirus proteins which assist in the initial stages (PM penetration and virion adsorption) of larval infections. Applicants' inability to identify cross-reacting proteins in the NPVs suggests that while these viruses may have proteins which are functionally related to the GV enhancing factors (1), they are unrelated in primary amino acid sequence.

The identified baculovirus enhancing proteins can be tentatively separated into 3 distinct groups based on molecular weight: 104Kd, 110Kd, and 80Kd. All of the research has concentrated on 2 enhancing factors from the same group, PuGV-H and TnGV (104Kd).

The absence of a protein in PuGV-O which does not cross-react to the VEF antiserum, confirms earlier reports indicating that PuGV-O does not contain an enhancing factor (6) and that differences exist in the capsular components of PuGV-H and PuGV-O (34,41).

It is important to note that the baculoviruses are just one of many insect pathogenic organisms that have evolved mechanisms, both behavioral and structural, to circumvent the PM (25) and Babesia microti, an intraerythrocytic piroplasm of the tick Ixodes dammini, has developed a complex "arrowhead" structure which secretes a series of digestive enzymes to enable passage through the PM (31).

The VEF and SF genes of the present invention can be used in engineering new viral pesticides with enhanced efficacy. For example, it can be used alone as biopesticide or in combination with known biological insecticides such as BT or with synthetic chemical insecticides. The gene product of this

invention can also be used to produce VEF or SF in any microbial production system, e.g. E. coli, Bacillus or Streptococcus. It can be introduced into a variety of hosts such as plants for protection against insects or microbes as biologically active agents.

The genes of this invention can be engineered to be expressed in transgenic plants and as insects feed on these plants, they would ingest a constant dose of the factor. While the exact effect of this on the insect is undetermined, it can be hypothesized that prolonged disruption of the peritrophic membrane (PM) may allow opportunistic microbes to infect and kill the insects. It was recently found that the viral factor increases the efficiency of Bt delta endotoxin by removing a major mechanical barrier--the PM.

The genes of the present invention have been found to play a significant role as a determinant of virulence at the initial stage of infection in insect hosts. Knowledge gained in cloning and sequencing the viral gene should prove useful in helping to unravel the mechanism(s) of enhanced virus infection by enhancement factors present within the occlusion body matrix.

Notwithstanding that reference has been made to particular preferred embodiments, it will be understood that the present invention is not to be construed as limited as such, but rather to the lawful scope of the appended claims. In other words, the subject invention includes not only the specific nucleotide sequences depicted herein, but also all equivalent nucleotide sequences coding for molecules with substantially the same biological activity of enhancing the infectivity of baculoviruses. The term "equivalent" is being used in ordinary patent usage here as denoting a nucleotide sequence which performs substantially as the nucleotide sequence identified herein to produce molecules with substantially the same biological activity in essentially the same kind of hosts. Within this definition are subfragments which have biological activity of enhancing the infectivity of baculoviruses.

Inasmuch as the protein, i.e., the gene product, of the present invention has been defined by means of deductive amino acid sequencing, c.f. Fig. 3, it is to be understood that for this particular protein, embraced herein, natural allelic variations exist and occur from individual to individual. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. All such allelic variations are included within the scope of the present invention.

BIBLIOGRAPHY

1. Derksen, A.G.S. and Granados, R.R. Virology 167: 242-250 (1988).
2. Peters, W. and Wiese, B. J. Insect Physio. 32:43-49 (1986).
3. Snyder, M., Sweatser, D., Young, R.A. and Davis, R.W. Meth. Enzym. 154:107-128 (1985).
4. Vlak, J.M. and Smith, G.E. J. Virol. 41:1118-1121 (1982).
5. Rohrmann, G. F. J. Gen. Virol. 67:1499-1513 (1986).
6. Tanada, Y. and Hukuhara, T. J. Invertebr. Pathol. 17:116-126 (1971).
7. Tanada, Y. and Hara, S. Nature 254:328-329 (1975).
8. Tanada, Y. J. Insect Pathol. 1:215-231 (1959).
9. Tanada, Y. Inoue, H., Hess, R.T., and Omi, E.M. J Inverebr. Pathol. 35:249-255 (1980).
10. Tanada, Y. J. Invertebr. Pathol. 45:125-138 (1985).
11. Yamamoto, T., Kita. H. and Tanada, Y. J. Gen. Virol. 45:371-381 (1979).
12. Maniatis, T., Fritsch, E.F. and Sambrook, J. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1982).
13. Hamm, J.J. and Paschke. J.D. J. Insect Pathol. 5:187-197 (1963).
14. Whitlock, V.H. J. Invertebr. Pathol. 23:70-75 (1974).
15. Granados, R.R. and Williams, K.A. The Biology of Baculoviruses, CRC Press, Fl. Vol I:89-108 (1986).
16. Miller, L.K. The Biology of Baculoviruses, CRC Press, Fl. Vol I:217-238 (1986).
17. Dwyer, K.G. & Granados, R.R. J. Virol. 62:1535-1542 (1988).
18. Jacques R.P. Can. Entomol. 120:575-580 (1988).
19. Sears, M.K. et al. J. Econ. Entomology 76:368-374 (Apr. 1983).
20. Blake, M.S., Johnston, K.H., Russel-Jones, G.J. & Gotschlich, E.C. Analyical Biochemistry 136, 175-179 (1984).
21. Gallo, L.G , Corsaro, B.G., Hughes, P.R. & Granados, R.R. Journal of Invertebrate Pathology 58:203-210 (1991).
22. Goto, C. Applied Entomology and Zoology 25, 135-137 (1990).
23. Hashimoto, Y., Corsaro, B.G. & Granados, R.R. Journal of General Virology 72, 2645-2651 (1991).
24. Hara, S., Tanada, Y. & Omi, E.M. Journal of Invertebrate Pathology 27, 115-124 (1976).

25. Huber, M., Cabib, E. & Miller, L.H. Proceedings of the National Academy of Sciences, U.S.A. 88,2807-2810 (1991).
26. Hughes, P.R. & Wood, H.A. Journal of Invertebrate Pathology 37, 154-159 (1981).
27. Hughes, P.R., Wood, H.A., Burand, J.P. & Granados, R.R. Journal of Invertebrate Pathology 43, 343-350 (1984).
28. Hughes, P.R., van Beek, N.A.M. & Wood, H.A. Journal of Invertebrate Pathology 48, 187-192 (1986).
29. Laemmli, U.K. Nature London 227,680-685 (1970).
30. Ooi, B.G., Rankin, C., & Miller, L.K. Journal of Molecular Biology 210,721-736 (1989).
31. Rudzinska, M.A., Spielman, A., Lewngrub, S., Piesman, J. & Karakshian, S. Cell and Tissue Research 221, 471-481 (1982).
32. Sanger, F., Nicklen, S. & Coalson, A.R. Proceedings of the National Academy of Sciences, U.S.A. 74, 5463-5467 (1977).
33. Tanada, Y. & Hukuhara, T. Journal of Invertebrate Pathology 12, 262-268 (1968).
34. Tanada, Y. & Watanabe, H. Journal of Invertebrate Pathology 18, 307-312 (1971).
35. Tanada, Y., Himeno, M. & Omi, E.M. Journal of Invertebrate Pathology 21, 31-40 (1973).
36. Tanada, Y., Hess, R.T. & Omi, E.M. Journal of Invertebrate Pathology 26, 99-104 (1975).
37. Tanada, Y., Hess, R.T., Omi, E.M. & Yamamoto, T. Microbios 37, 87-93 (1983).
38. Towbin, H., Staehelin, R. & Gordon, J. Proceedings of the National Academy of Sciences, U.S.A. 76, 4350-4354 (1979).
39. Uchima, K., Harvey, J.P., Omi, E.M. & Tanada, Y.A. Insect Biochemistry 18, 645-650 (1988).
40. Vieira, J. & J. Messing. Gene 19, 259-286 (1982).
41. Yamamoto, T. & Tanada, Y. Journal of Invertebrate Pathology 32, 158-170 (1978).
42. Yamamoto, T. & Tanada, Y. Virology 107, 434-440 (1980).
43. Zhu, Y., Hukuhara, T. & Tamura, K. Journal of Invertebrate Pathology 54, 49-56 (1989).

INFORMATION FOR SEQ ID NO:1

(i)	(A)	LENGTH: 3556 basepairs	
	(B)	TYPE: Nucleic with corresponding amino acid sequence (435-3140)	
	(C)	STRANDEDNESS: Doublestranded	
	(D)	TOPOLOGY: Circular	
(ii)		MOLECULE TYPE: Genomic DNA	
(iii)		HYPOTHETICAL: No	
(iv)		ANTISENSE: No	
(v)		FRAGMENT TYPE: N/A	
(vi)	(A)	ORIGINAL SOURCE: *Trichoplusia ni* granulosis virus	
	(B)-(F)	Not applicable	
(vii)	(A)	LIBRARY: Lambda GT11	
	(B)	CLONE: HindIII-M	
(viii)	(A)	CHROMOSOME/SEGMENT: N/A	
	(B)	MAP POSITION: 92.2 - 94.2	
	(C)	UNITS: genome percent	

(ix) FEATURE

1.	(A)	Mature protein				
	(B)	435 bp to 3140 bp				
	(C)	Experimentally				
	(D)	Degrades special Peritrophic Membrane proteins Binds to midgut brush border				
2.	(A)	Baculovirus very late promoter				
	(B)	427 to 432 bp				
	(C)	N/A				
	(D)	N/A				
3.	(A)	Possible glycosylation sites				
	(B)	Site 1	65	to	67	amino acids
		Site 2	265	to	267	amino acids
		Site 3	306	to	308	amino acids
		Site 4	339	to	341	amino acids
		Site 5	349	to	351	amino acids
		Site 6	540	to	542	amino acids
		Site 7	594	to	596	amino acids
		Site 8	595	to	597	amino acids
		Site 9	621	to	623	amino acids
		Site 10	642	to	644	amino acids
		Site 11	683	to	685	amino acids
		Site 12	698	to	700	amino acids

(C) Experimental
(D) Not known

(xi) SEQUENCE DESCRIPTION

SEQUENCE ID NUMBER: 1
SEQUENCE TYPE: Nucleotide with corresponding protein
SEQUENCE LENGTH: 3556 base pairs

```
CAGCGCGAAA ACGGTTGGTG CCAATTACTG GTATATTGCT ATGATCGAGT GACGTCATAA      60
GGGTCGATTC GCGACGGTCT CCCACGTGGC CTTCCATTGA GGTTTACGTG TTTGTGTATG     120
CGTGCGAGTG TTTTTATAAC CCAAAAACTC AGCCACACCG TGTCCACCGT ACATATACTT     180
GTCCTTTTCC AATTCCACAA TCCAAATTTC CGCAGAAACT CCTCCAATGT TGCACGATTT     240
TTTTACAAGA GTCATTTTGC ACGTTTACAA GAAATTTATT ACAAGATTAG CTGCTTGTGA     300
TAAAGGTCTG CACGAGATGA GATTCAAATA CGTAATGAGA ATTGCGTGAT TTGCACGAGT     360
TTATATAGCA TAATTTGCTA GGAATGTCTG TTGGTTTGTG ATGTTTAGGT GTTCGCTGCA     420
TTAATTATAA GACT ATG TCG TAC AAA GTG ATT GTA CCC GCT ACC GTG CTA        470
                MET Ser Tyr Lys Val Ile Val Pro Ala Thr Val Leu
                  1               5                  10

CCG CCG TGG CTC AGA GTC GGT GAG AAT TGG ATA TTC GCA AGA CAC AGA       518
Pro Pro Trp Leu Arg Val Gly Glu Asn Trp Ile Phe Ala Arg His Arg
          15                  20                  25

CGC ACC GAG GTG GGA GTC GTT CTA CCG GCG AAC ACG AAA TTT CGT GTA       566
Arg Thr Glu Val Gly Val Val Leu Pro Ala Asn Thr Lys Phe Arg Val
      30                  35                  40

CGA GCA GAT TTC TCT AGG GCC GGC TTC ACC CGA CCC GTA ATA GTG CGC       614
Arg Ala Asp Phe Ser Arg Ala Gly Phe Thr Arg Pro Val Ile Val Arg
  45                  50                  55                  60

CTC TTG AAC AAC AAC CGT AGC ACT GAA CGA GAA ATC AAC TTG AAC AAC       662
Leu Leu Asn Asn Asn Arg Ser Thr Glu Arg Glu Ile Asn Leu Asn Asn
                65                  70                  75

GAC CAA TGG ATG GAG GTG GAG CAT GCG CAC GAG AGT GTG CCT TTC GTA       710
Asp Gln Trp Met Glu Val Glu His Ala His Glu Ser Val Pro Phe Val
            80                  85                  90

GAT TGG CTG GTG GGC GAA AAG AAC ACT ATG GCC GAA GTG TAT TTT GAA       758
Asp Trp Leu Val Gly Glu Lys Asn Thr MET Ala Glu Val Tyr Phe Glu
          95                  100                 105

ATC GAC GGA CCA CAC ATA CCG CTA CCC GTG TAC GTG TTC AAC ACG AGA       806
Ile Asp Gly Pro His Ile Pro Leu Pro Val Tyr Val Phe Asn Thr Arg
      110                 115                 120

CCC GTC GAA CAC TTT AAG AGC GAG TAT CGC CAA AGT TCG TCT GGC TAC       854
Pro Val Glu His Phe Lys Ser Glu Tyr Arg Gln Ser Ser Ser Gly Tyr
125                 130                 135                 140

TGC TTT CTA TAT TTG GAC CTG GTC TGT ATG TTG GTA CCG CCC GCT AGC       902
Cys Phe Leu Tyr Leu Asp Leu Val Cys MET Leu Val Pro Pro Ala Ser
```

```
                    145                 150                 155

AAA AAC GCT TTA TTG GAC GTG AAC ATT TTC GAG CTT CAT CAA TTT TAT      950
Lys Asn Ala Leu Leu Asp Val Asn Ile Phe Glu Leu His Gln Phe Tyr
            160                 165                 170

AAC GAA ATC ATT AAT TAC TAT GAT GAC CTG TGC GGC TTG GTC GAG GAT      998
Asn Glu Ile Ile Asn Tyr Tyr Asp Asp Leu Cys Gly Leu Val Glu Asp
        175                 180                 185

CCA TAC GCA GAC ACT GTC GAT TCG AAT TTA CCC AAC AAG GCT GCT TTC      1046
Pro Tyr Ala Asp Thr Val Asp Ser Asn Leu Pro Asn Lys Ala Ala Phe
    190                 195                 200

GTG AAA GCT GAT GCT GGC GGT CCG GGT GGT GCG TAT TAT GGA CCA TTT      1094
Val Lys Ala Asp Ala Gly Gly Pro Gly Gly Ala Tyr Tyr Gly Pro Phe
205                 210                 215                 220

TGG ACG GCA CCG GCG AGC TCA AAC CTT GGT GAT TAC CTC AGA ATA TCG      1142
Trp Thr Ala Pro Ala Ser Ser Asn Leu Gly Asp Tyr Leu Arg Ile Ser
                225                 230                 235

CCG ACC AAC TGG ATG GTA ATT CAC GAG CTG GGT CAT GCA TAC GAT TTT      1190
Pro Thr Asn Trp MET Val Ile His Glu Leu Gly His Ala Tyr Asp Phe
            240                 245                 250

GTG TTT ACC GTC AAC ACT ATA CTC ATT GAA ATT TGG AAC AAC TCT TTA      1238
Val Phe Thr Val Asn Thr Ile Leu Ile Glu Ile Trp Asn Asn Ser Leu
        255                 260                 265

TGC GAT CGC ATC CAA TAC AAG TGG ATG AAC AAA ATT AAA AGA CAA CAA      1286
Cys Asp Arg Ile Gln Tyr Lys Trp MET Asn Lys Ile Lys Arg Gln Gln
    270                 275                 280

CTG GCT CGC GTC TAT GAA AAT AGA CGA CCG CAG AAA GAG GCG ACC ATT      1334
Leu Ala Arg Val Tyr Glu Asn Arg Arg Pro Gln Lys Glu Ala Thr Ile
285                 290                 295                 300

CAG GCG CTG ATC GAC AAT AAC AGC CCG TTC GAT AAT TGG GGC TTT TTT      1382
Gln Ala Leu Ile Asp Asn Asn Ser Pro Phe Asp Asn Trp Gly Phe Phe
                305                 310                 315

GAG AGG CTG ATA ATA TTC ACG TGG CTG TAC AAC CCG CAA AGA GGA CTA      1430
Glu Arg Leu Ile Ile Phe Thr Trp Leu Tyr Asn Pro Gln Arg Gly Leu
            320                 325                 330

GAC ACA TTG CGT AAC ATC AAC CAT TCG TAC AGG GTG CAC GCC ACC CGC      1478
Asp Thr Leu Arg Asn Ile Asn His Ser Tyr Arg Val His Ala Thr Arg
        335                 340                 345

AAC TCT TCT ATA CCG TAC CCG CAA ATA TGG TCA TGG CTA ACG ACT TCT      1526
Asn Ser Ser Ile Pro Tyr Pro Gln Ile Trp Ser Trp Leu Thr Thr Ser
    350                 355                 360

GCT TAC GAC AAC TTT TGG TTA TAT TTT AAT TTG GTA GGC GTG TAC CCG      1574
```

```
Ala Tyr Asp Asn Phe Trp Leu Tyr Phe Asn Leu Val Gly Val Tyr Pro
365                 370                 375                 380

GCA GAC TTT TAC GTA AAC GAA CAC AAC AAA GTT GTT CAT TTC AAT CTA      1622
Ala Asp Phe Tyr Val Asn Glu His Asn Lys Val Val His Phe Asn Leu
                385                 390                 395

CAC TTG AGA GCT TTG GCG TTG GGG CAG AGT GTG CGT TAT CCC ATT AAA      1670
His Leu Arg Ala Leu Ala Leu Gly Gln Ser Val Arg Tyr Pro Ile Lys
            400                 405                 410

TAT ATA ATT ACA GAC TTT GAT CTG GTG AGC AAA AAC TAC GAC ATT AAA      1718
Tyr Ile Ile Thr Asp Phe Asp Leu Val Ser Lys Asn Tyr Asp Ile Lys
        415                 420                 425

CAG TAT TTA GAG AGT AAT TTC GAT CTG GTT ATA CCA GAA GAA TTG CGG      1766
Gln Tyr Leu Glu Ser Asn Phe Asp Leu Val Ile Pro Glu Glu Leu Arg
    430                 435                 440

CAG ACC GAT TTG TTG GCG GAC GTG AGG GTG GTT TGT GTG ATT GAC GAT      1814
Gln Thr Asp Leu Leu Ala Asp Val Arg Val Val Cys Val Ile Asp Asp
445                 450                 455                 560

CCG TCG CAG ATT GTG GGC GAA CCG TTT AGC GTG TAC GAC GGG AAC GAG      1862
Pro Ser Gln Ile Val Gly Glu Pro Phe Ser Val Tyr Asp Gly Asn Glu
                465                 470                 475

CGA GTG TTC GAG AGT ACG GTG GCC ACG GAC GGA AAC ATG TAT CTG GTG      1910
Arg Val Phe Glu Ser Thr Val Ala Thr Asp Gly Asn MET Tyr Leu Val
            480                 485                 490

GGC GTG GGT CCG GGA GTG TAC ACG TTG CGT GCG CCA CGC GGC AAA AAC      1958
Gly Val Gly Pro Gly Val Tyr Thr Leu Arg Ala Pro Arg Gly Lys Asn
        495                 500                 505

AAA CGC TAC AAA CTC CAT TTG GCA CAT TCG CCC AGA GAG CCC GTT CAT      2006
Lys Arg Tyr Lys Leu His Leu Ala His Ser Pro Arg Glu Pro Val His
    510                 515                 520

CCG GCC AAC GAC CAC ATG TAT CTG CTC GTG ACG TAT CCC TAC TAC AAT      2054
Pro Ala Asn Asp His MET Tyr Leu Leu Val Thr Tyr Pro Tyr Tyr Asn
525                 530                 535                 540

CAA ACG TTG ACA TAC ACA CCG TAC GTA AAT TCT GAC CTA GCC GTC GAC      2102
Gln Thr Leu Thr Tyr Thr Pro Tyr Val Asn Ser Asp Leu Ala Val Asp
                545                 550                 555

ATG GCT CAT TTG TTC GGC AGC AAC GAT CGT AGG TAT GTA GCC ACG ATA      2150
MET Ala His Leu Phe Gly Ser Asn Asp Arg Arg Tyr Val Ala Thr Ile
            560                 565                 570

TAT TTC AAT CCA TTC GAA CAA ACA GTC ACC GTA CAT CTA AAC AAT ATT      2198
Tyr Phe Asn Pro Phe Glu Gln Thr Val Thr Val His Leu Asn Asn Ile
        575                 580                 585
```

```
CGT GCC GGT CGT GAA AAC AAC ACT ACC CTG TAC TTT GAA ATG GTA ATT          2246
Arg Ala Gly Arg Glu Asn Asn Thr Thr Leu Tyr Phe Glu MET Val Ile
    590                 595                 600

AGC AAC CCG TTC AAC GGG CAG AGC CAA ACT TTC ACT ATA CTC GAA GAC          2294
Ser Asn Pro Phe Asn Gly Gln Ser Gln Thr Phe Thr Ile Leu Glu Asp
605                 610                 615                 620

AAT CCC ACT TTA CGA CAA GGC TAC TAC AAA TTT GAC GTG GTC ACG TAC          2342
Asn Pro Thr Leu Arg Gln Gly Tyr Tyr Lys Phe Asp Val Val Thr Tyr
                625                 630                 635

AGC TCC ATA AGG CTG AAT ATG AGC GTC GCG GGT CGG CTA TTA TTT CGG          2390
Ser Ser Ile Arg Leu Asn MET Ser Val Ala Gly Arg Leu Leu Phe Arg
            640                 645                 650

CGA TAC ATT TTT GCC GGA GGT ACC ACC ACG CTG ACC ATG TTC CCA AAT          2438
Arg Tyr Ile Phe Ala Gly Gly Thr Thr Thr Leu Thr MET Phe Pro Asn
        655                 660                 665

CAA GTA CTT GAG CCC AAT TTG TTT CCA GAC GGT TCC GCC TTG AAT AGG          2486
Gln Val Leu Glu Pro Asn Leu Phe Pro Asp Gly Ser Ala Leu Asn Arg
    670                 675                 680

ACA TTG GCA CGA CTA AGA GAA CAG GCC GCC TTC CTA GAT AAT TAT TCA          2534
Thr Leu Ala Arg Leu Arg Glu Gln Ala Ala Phe Leu Asp Asn Tyr Ser
685                 690                 695                 700

CAA CTT ATG TAT ATT GAA AAC GAG TTG CGC GAC ACG ATT TAT TTG GCC          2582
Gln Leu MET Tyr Ile Glu Asn Glu Leu Arg Asp Thr Ile Tyr Leu Ala
                705                 710                 715

TCC CAG TTG GTA GAT CCT GCG TCA GAC GAA TTT GTA AAG TAT TAT CCA          2630
Ser Gln Leu Val Asp Pro Ala Ser Asp Glu Phe Val Lys Tyr Tyr Pro
            720                 725                 730

GAC TAC TTC AGA GAT CCG CAC ACG TAC GTG TAC TTG TTT CGT TTC AGA          2678
Asp Tyr Phe Arg Asp Pro His Thr Tyr Val Tyr Leu Phe Arg Phe Arg
        720                 740                 745

GGT CTG GGT GAT TTC GTG TTA TTA GAC TTG CAG ATT GTA CCA TTG CTA          2726
Gly Leu Gly Asp Phe Val Leu Leu Asp Leu Gln Ile Val Pro Leu Leu
    750                 755                 760

AAT TTG GCC ACT GTA CGT ATA GCC AAC ATC CAA AAC GGT CCC CAC TCG          2774
Asn Leu Ala Thr Val Arg Ile Ala Asn Ile Gln Asn Gly Pro His Ser
765                 770                 775                 780

TAC TTC GAT ACT TTG TAT TTT AAA GTG GAG TTG CGC GAC ACA AAC GGT          2822
Tyr Phe Asp Thr Leu Tyr Phe Lys Val Glu Leu Arg Asp Thr Asn Gly
                785                 790                 795

GCG ATT GTG TTT TCG TAT TCG CGC CGT GGC AAC GAG CCG ATG ACA CCC          2870
Ala Ile Val Phe Ser Tyr Ser Arg Arg Gly Asn Glu Pro MET Thr Pro
            800                 805                 810
```

```
GAA CAC CAT AAA TTT GAA GTG TAC AGT GGT TAC ACC GTA GAA TTG TTC        2918
Glu His His Lys Phe Glu Val Tyr Ser Gly Tyr Thr Val Glu Leu Phe
        815                 820                 825

ATG CGG GAA CCC GGT AAT CGA TTA CAA TTG ATT GTG AAC AAA ATG CTT        2966
MET Arg Glu Pro Gly Asn Arg Leu Gln Leu Ile Val Asn Lys MET Leu
    830                 835                 840

GAC ACA GCG TTG CCG TCT ACT CAA AAC ATT TTC GCT CGC ATC ACC GAC        3014
Asp Thr Ala Leu Pro Ser Thr Gln Asn Ile Phe Ala Arg Ile Thr Asp
845                 850                 855                 860

ACT CAA TTA GTG GTG GGG GAT ACG AGC ATT GAA GAT AAC CTT GTA ACG        3062
Thr Gln Leu Val Val Gly Asp Thr Ser Ile Glu Asp Asn Leu Val Thr
                865                 870                 875

AGT ATT AAT GTA GAT TGT GGC GAC GAC GAC AAC CAA AAG ATA AGA GTT        3110
Ser Ile Asn Val Asp Cys Gly Asp Asp Asp Asn Gln Lys Ile Arg Val
            880                 885                 890

GTG GAA ACG TTA AAA ATG ATA GCG TTC TAA TAACGTTCAA CAGTCAGTTA          3160
Val Glu Thr Leu Lys MET Ile Ala Phe
        895                 900

TCGACTGTCG CCGCGACGAC ATGACACTGG TGGGTGTAGT AGTTTGCGTG CTGTTGTTAT      3220
CGTCTGTAGA CGGTTATTCG TTTTATTCGT CGATTGAAGC CCTGCTTTTG AACGATCGCA      3280
CACAACTTTG CATAGGCGAC TGTTACGAAC GCAATGGCCA GCATTTGTGT GCCAGCACGT      3340
GGTCGGGATC AGAGTCTCGG TGCATAAGTG TTTTCAACAA GACCAAACAC TATCGTACGG      3400
AGACTAACGG AAAATGCATA AGTAACTGTG CCAACTTCAA CAACTACGCC CACGAATGGT      3460
GTGCCGTGTC CCGGTCGAAA TGGGGCCGTT GCAGCAGACG ACTGGCGCTC ACAGCGACAC      3520
GAACACACGC CACCCACAAC AAGTTCAAGA CATGTG                                3556
```

Claims

1. A purified baculovirus protein designated as an enhancin and found in granulosis viruses whithin the viral occlusion body. said viruses being isolated from one of the following families of Lepidoptera Arctiidae, Noctuidae, Pieridae and Tortricidae, said protein being characterized by having a disruptive effect on the insect peritrophic membrane proteins by interacting with the midgut epithelium in such a manner as to permit the increased adsorption, penetration, and uptake of virus particles by midgut cells with a concomitant increase in host mortality.

2. An enhancin of claim 1 having a molecular weight between 80 Kd and 110 Kd.

3. The enhancin of claim 1 having molecular weight of 104 Kd.

4. An isolated and purified DNA from a granulosis virus comprising a DNA encoding the 901 amino acid residue polypeptide as shown in figure 3 and which polypeptide possesses the biological activity of enhancing baculovirus infectivity.

5. The DNA of claim 4 comprising the DNA of figure 3 from base +1 to base +2703 which is an open reading frame of 2703 base pairs encoding a protein with a molecular weight of 104 Kd.

6. The DNA of claim 4 comprising an allele encoding the 901 amino acid polypeptide.

7. An insecticide comprising a polypeptide characterized by having the amino acid and nucleotide sequence of figure 3.

8. A biopesticide comprising an enhancin of claim 1.

9. A composition comprising an enhancin of claim 1 and a pesticide.

10. A toxicant composition comprising an enhancin of claim 1 and a biological insecticide.

11. A toxicant composition comprising an enhancin of claim 1 and a synthetic chemical insecticide.

12. A microbial or cellular protein system for the production of an enhancin of claim 1.

13. The microbial system of claim 12 in which the microorganisms are selected from the group consisting of E. coli, Bacillus, Streptococcus, and a Baculovirus expression system.

14. Process of introducing an enhancin of claim 1 into plants for protection against insects.

15. Process of introducing an enhancin into microbes useful as biologically active agents.

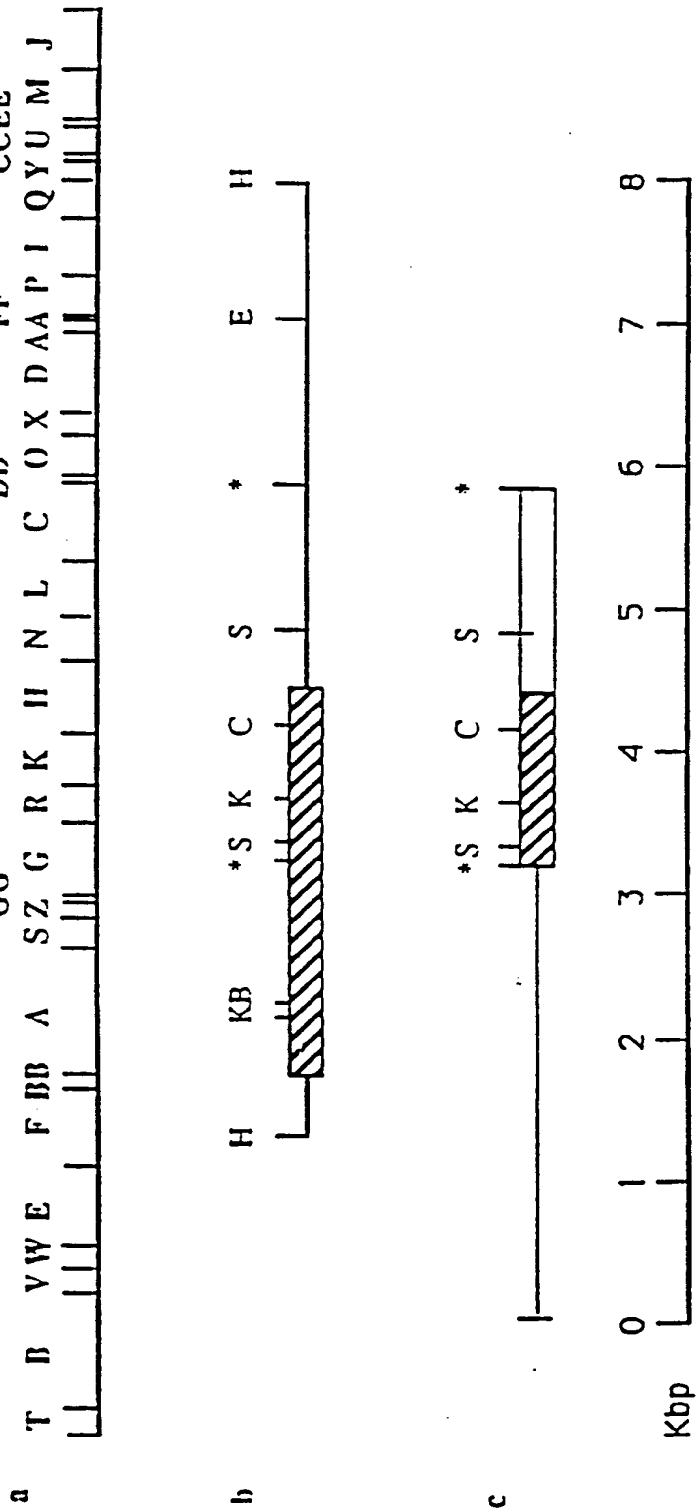

a
T B VW E F BB A SZ G R K H N L C DD O X D AA P I FF Q Y U M J CCEE
b
H KB *S K C S * E H
c
*S K C S *
0 1 2 3 4 5 6 7 8
Kbp

FIG. 1

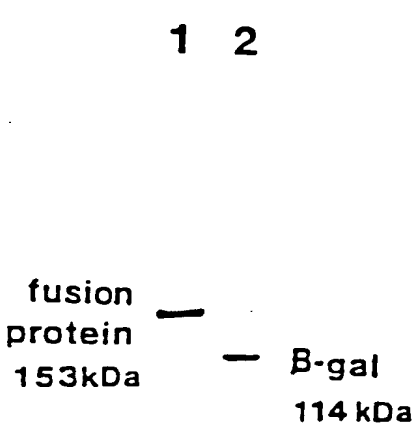


FIG. 2


CAGCGCGAAAACGGTTGGTGCCAATTACTGGTATATTGCTATGATCGAGTGACGTCATAAGGGTCGATTCGCGACGGTCTCCCACGTGGCCTTCCATTGAGGTTTACGTGTTTGTGTATG -315
CGTGCGAGTGTTTTTATAACCCAAAAACTCAGCCACACCGTGTCCACCGTACATATACTTGTCCTTTTCCAATTCCACAATCCAAATTTCCGCAGAAACTCCTCCAATGTTGCACGATTT -195
TTTTACAAGAGTCATTTTGCACGTTTACAAGAAATTTATTACAAGATTAGCTGCTTGTGATAAAGGTCTGCACGAGATGAGATTCAAATACGTAATGAGAATTGCGTGATTTGCACGAGT -75
TTATATAGCATAATTTGCTAGGAATGTCTGTTGGTTTGTGATGTTTAGGTGTTCGCTGCATTAATTATAAGACTATGTCGTACAAAGTGATTGTACCCGCTACCGTCGTACCGCCGTGGC +46
M S Y K V I V P A T V V P P W
TCAGAGTCGGTGAGAATTGGATATTCGCAAGACACAGACGCACCGAGGTGGGAGTCGTTCTACCGGCGAACACGAAATTTCGTGTACGAGCAGATTTCTCTAGGGCCGGCTTCACCCGAC +166
L R V G E N W I F A R H R R T E V G V V L P A N T K F R V R A D F S R A G F T R
CCGTAATAGTGCGCCTCTTGAACAACAACCGTAGCACTGAACGAGAAATCAACTTGAACAACGACCAATGGATGGAGGTGGAGCATGCGCACGAGAGTGTGCCTTTCGTAGATTGGCTGG +276
P V I V R L L N N N S S T E R E I N L N N D Q W M E V E H A H E S V P F V D W L
1
TGGGCGAAAAGAACACTATGGCCGAAGTGTATTTTGAAATCGACGGACCACACATACCGCTACCCGTGTACGTGTTCAACACGAGACCCGTCGAACACTTTAAGAGCGAGTATCGCCAAA +396
V G E K N T M A E V Y F E I D G P H I P L P V Y V F N T R P V E H F K S E Y R Q
GTTCGTCTGGCTACTGCTTTCTATATTTGGACCTGGTCTGTATGTTGGTACCGCCCGCTAGCAAAAACGCTTTATTGGACGTGAACATTTTCGAGCTTCATCAATTTTATAACGAAATCA +516
S S S G Y C F L Y L D L V C M L V P P A S K N A L L D V N I F E L H Q F Y N E I
TTAATTACTATGATGACCTGTGCGGCTTGGTCGAGGATCCATACGCAGACACTGTCGATTCGAATTTACCCAACAAGGCTGCTTTCGTGAAAGCTGATGCTGGCGGTCCCGGTGGTGGGT +636
I N Y Y D D L C G L V E D P Y A D T V D S N L P N K A A F V K A D A G G P G G A
ATTATGGACCATTTTCGACGGCACCGGCGAGCTCAAACCTTGGTGATTACCTCAGAATATCGCCGACCAACTGGATGGTAATTCACGAGCTGGGTCATGCATACGATTTTGTGTTTACCG +756
Y Y G P F W T A P A S S N L G D Y L R I S P T N W M V I H E L G H A Y D F V F T
TCAACACTATACTCATTGAAATTTGGAACAACTCTTTATGCGATCGCATCCAATACAAGTGGATGAACAAAATTAAAAGACAACAACTGGCTCGCGTCTATGAAAATACACCACCCCAGA +876
V N T I L I E I W N N N S L C D R I Q Y K W M N K I K R Q Q L A R V Y E N R R P Q
2
AAGAGGCGACCATTCAGGCGCTGATCGACAATAACAGCCCGTTCGATAATTGGGGCTTTTTTGAGAGGCTGATAATATTCACGTGGCTGTACAACCCGCAAAGAGGACTAGACACATTGC +996
K E A T I Q A L I D N N S P F D N W G F F E R L I I F T W L Y N P Q R G L D T L
3
GTAACATCAACCATTCGTACAGGGTGCACGCCACCCGCAACTCTTCTATACCGTACCCGCAAATATGGTCATGGCTAACGACTTCTGCTTACGACAACTTTTGGTTATATTTTAATTTGG +1116
R N I N H S Y R V H A T R N S S I P Y P Q I W S W L T T S A Y D N F W L Y F N L
4 5
TAGGCGTGTACCCGGCAGACTTTTACGTAAACGAACACAACAAAGTTGTTCATTTCAATCTACACTTGAGAGCTTTGGCGTTGGGGCAGAGTGTGCGTTATCCCATTAAATATATAATTA +1236
V G V Y P A D F Y V N E H N K V V H F N L H L R A L A L G Q S V R Y P I K Y I I
CAGACTTTGATCTGGTGAGCAAAAACTACGACATTAAACAGTATTTAGAGAGTAATTTCGATCTGGTTATACCAGAAGAATTGCGGCAGACCGATTTGTTGGCGGACGTCAGCGTGGTTT +1356
T D F D L V S K N Y D I K Q Y L E S N F D L V I P E E L R Q T D L L A D V R V V
GTGTGATTGACGATCCGTCGCAGATTGTGGGCGAACCGTTTAGCGTGTACGACGGGAACGAGCGAGTGTTCGAGAGTACGGTGGCCACGGACGGAAACATGTATCTGGTGGGCGTGGGTC +1476
C V I D D P S Q I V G E P F S V Y D G N E R V F E S T V A T D G N M Y L V G V G

FIG. 3

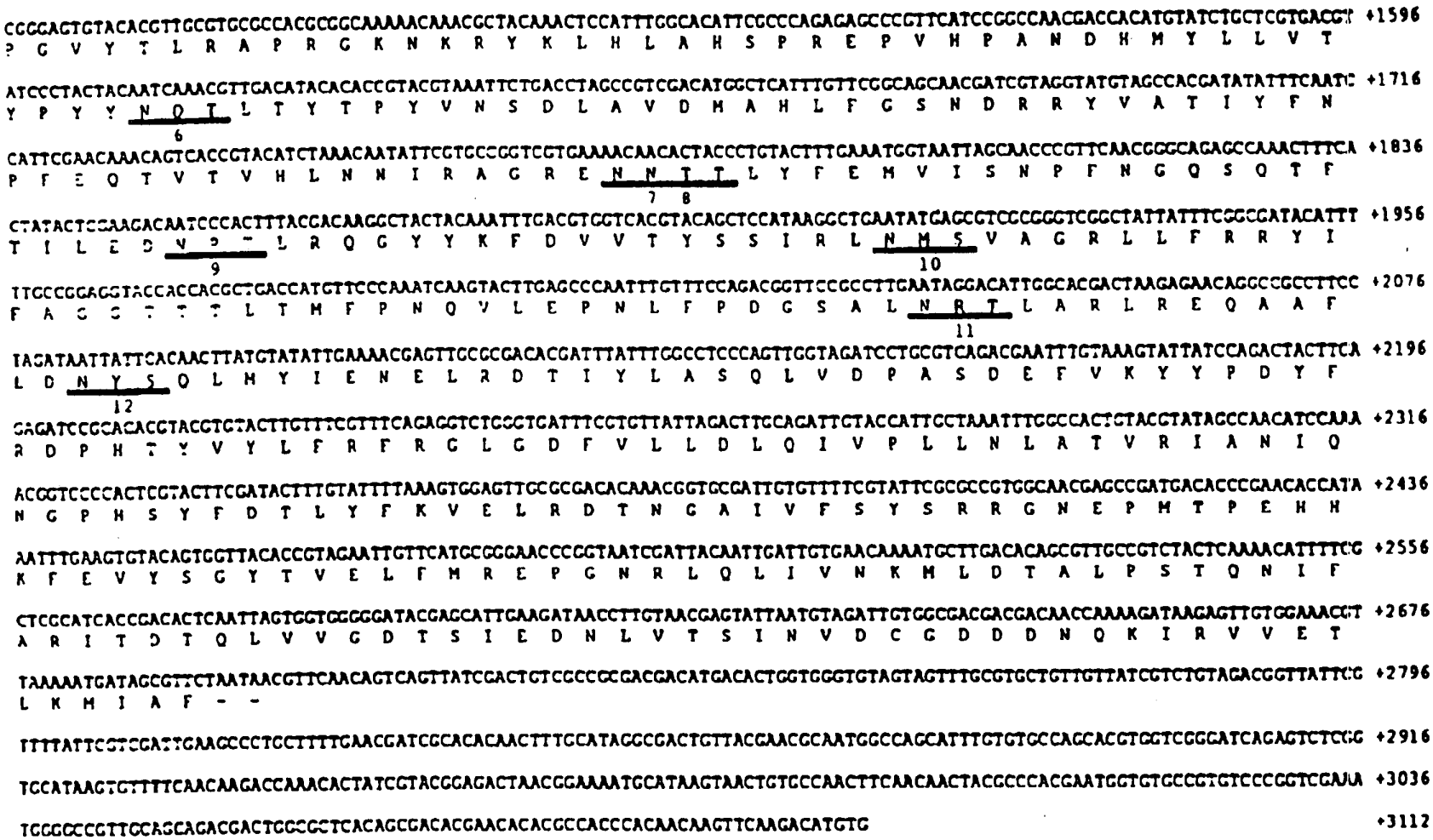


FIG. 3 (continued)

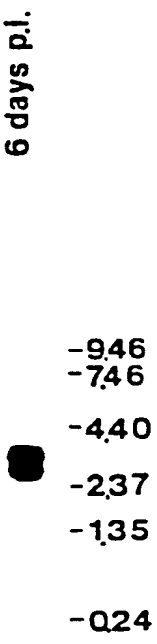


FIG. 4

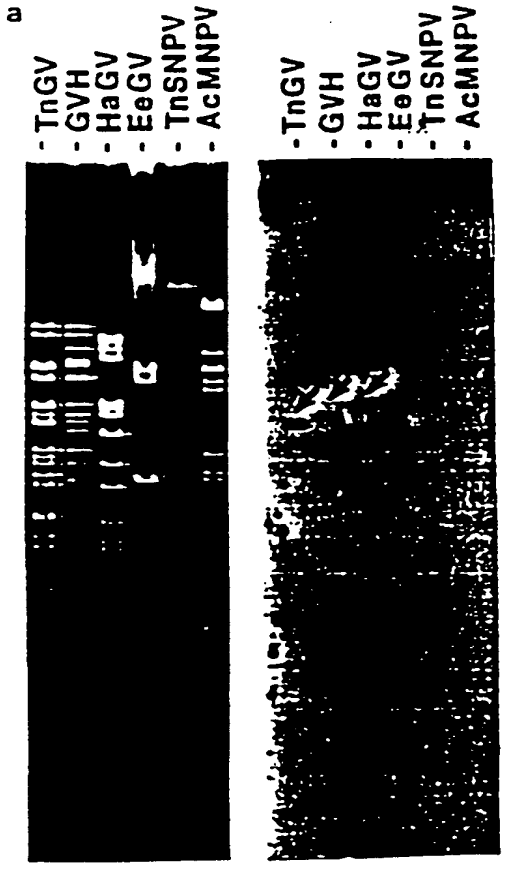

a
- TnGV
- GVH
- HaGV
- EeGV
- TnSNPV
- AcMNPV
- TnGV
- GVH
- HaGV
- EeGV
- TnSNPV
- AcMNPV

FIG. 5a

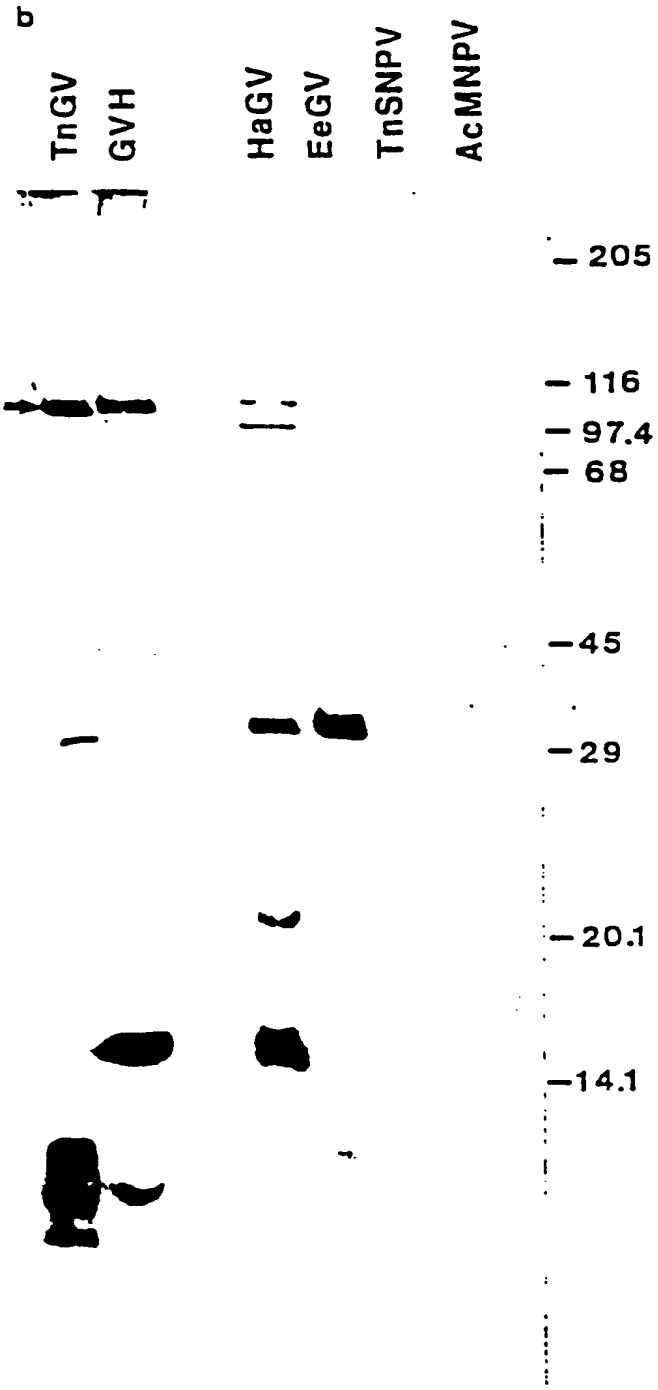


FIG 5b

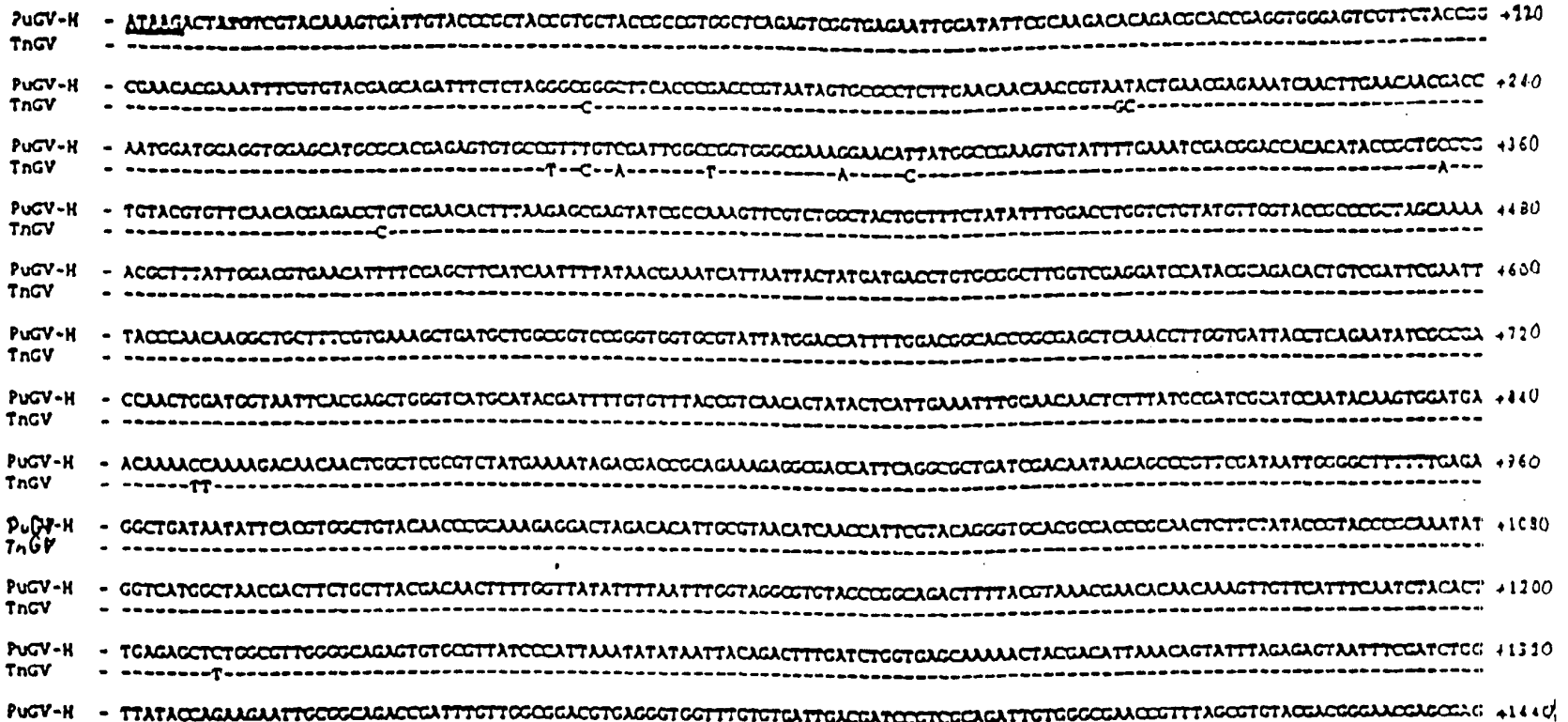


FIG. 6

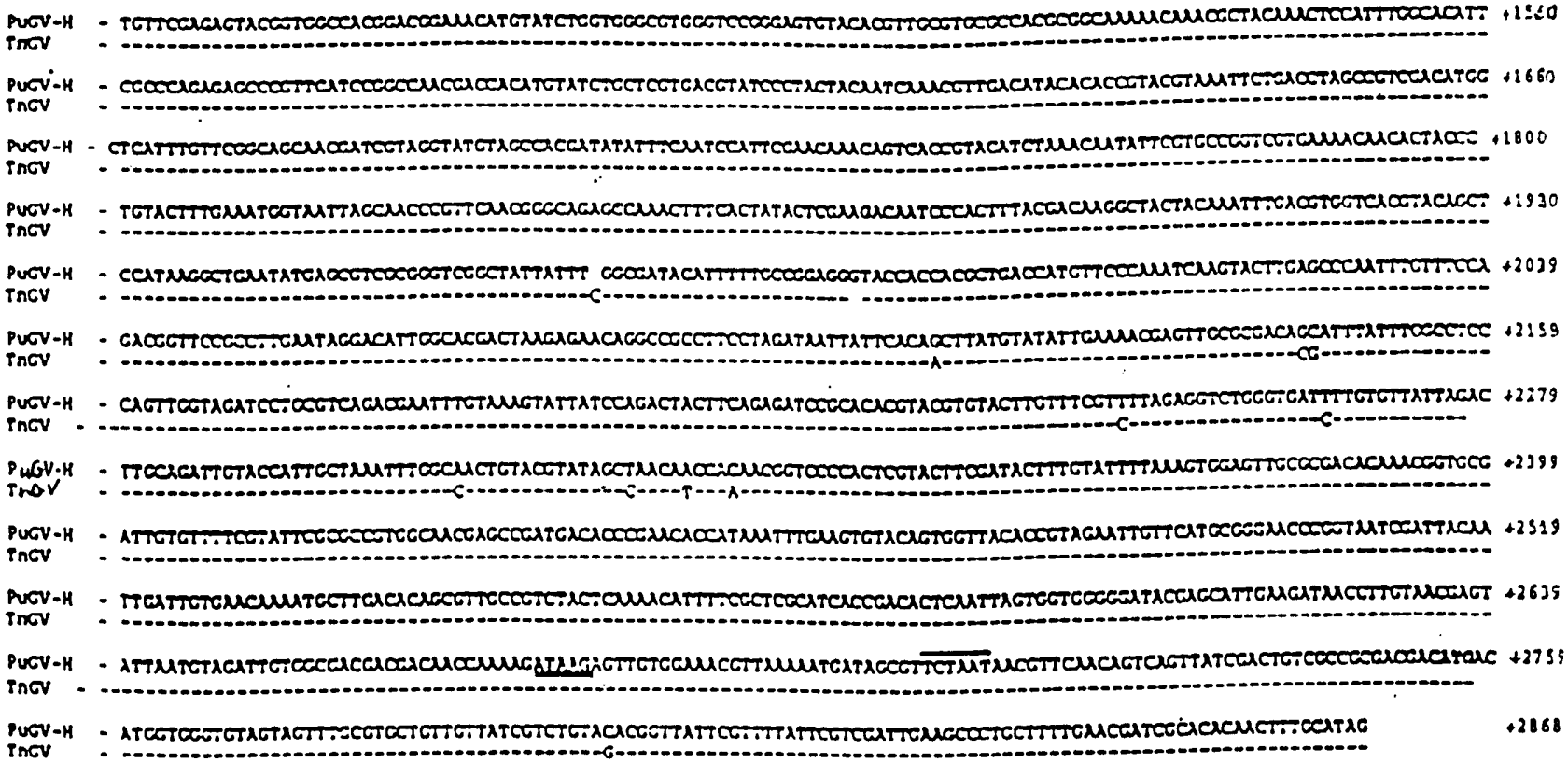


FIG. 6 (continued)

Fig. 7

```
PuGV-H - MSYKVIVPATVVPPWLRVGENWIFARHRRTEVGVVLPANTKFRVRADFSRAGFTRPVIVR -60
TnGV   - ------------------------------------------------------------

PuGV-H - LLNNNRNTEREINLNNDQWMEVEHAHESVPFVDWPVGERNIMAEVYFEIDGPHIPLPVYV -120
TnGV   - ------S---------------------------L---K-T-------------------

PuGV-H - FNTRPVEHFKSEYRQSSSGYCFLYLDLVCMLVPPASKNALLDVNIFELHQFYNEIINYYD -180
TnGV   - ------------------------------------------------------------

PuGV-H - DLCGLVEDPYADTVDSNLPNKAAFVKADAGGPGGAYYGPFWTAPASSNLGDYLRISPTNW -240
TnGV   - ------------------------------------------------------------

PuGV-H - MVIHELGHAYDFVFTVNTILIEIWNNSLCDRIQYKWMNKTKRQQLARVYENRRPQKEATI -300
TnGV   - ----------------------------------------I-------------------

PuGV-H - QALIDNNSPFDNWGFFERLIIFTWLYNPQRGLDTLRNINHSYRVHATRNSSIPYPQIWSW -360
TnGV   - ------------------------------------------------------------

PuGV-H - LTTSAYDNFWLYFNLVGVYPADFYVNEHNKVVHFNLHLRALALGQSVRYPIKYIITDFDL -420
TnGV   - ------------------------------------------------------------

PuGV-H - VSKNYDIKQYLESNFDLVIPEELRQTDLLADVRVVCVIDDPSQIVGEPFSVYDGNERVFE -480
TnGV   - ------------------------------------------------------------

PuGV-H - STVATDGNMYLVGVGPGVYTLRAPRGKNKRYKLHLAHSPREPVHPANDHMYLLVTYPYYN -540
TnGV   - ------------------------------------------------------------

PuGV-H - QTLTYTPYVNSDLAVDMAHLFGSNDRRYVATIYFNPFEQTVTVHLNNIRAGRENNTTLYF -600
TnGV   - ------------------------------------------------------------

PuGV-H - EMVISNPFNGQSQTFTILEDNPTLRQGYYKFDVVTYSSIRLNMSVAGRLLFGDTFLPEGT -660
TnGV   - ----------------------------------------------------RRYIFAG--

PuGV-H - TTLTMFPNQVLEPNLFPDGSALNRTLARLREQAAFLDNYSQLMYIENELRDSIYLASQLV -720
TnGV   - ------------------------------------------------T-------- ..

PuGV-H - DPASDEFVKYYPDYFRDPHTYVYLFRFRGLGDFVLLDLQIVPLLNLATVRIANNHNGPHS -780
TnGV   - ----------------------------------------------IQ-----

PuGV-H - YFDTLYFKVELRDTNGAIVFSYSRRGNEPMTPEHHKFEVYSGYTVELFMREPGNRLQLIV -840
TnGV   - ------------------------------------------------------------

PuGV-H - NKQLDTALPSTQNIFARITDTQLVVGDTSIEDNLVTSINVDCGDDDNQKIRVVETLKMIA -900
TnGV   - ------------------------------------------------------------

PuGV-H - FZ -902
TnGV   - --
```

Fig. 8

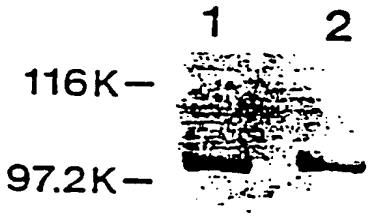


Fig 9

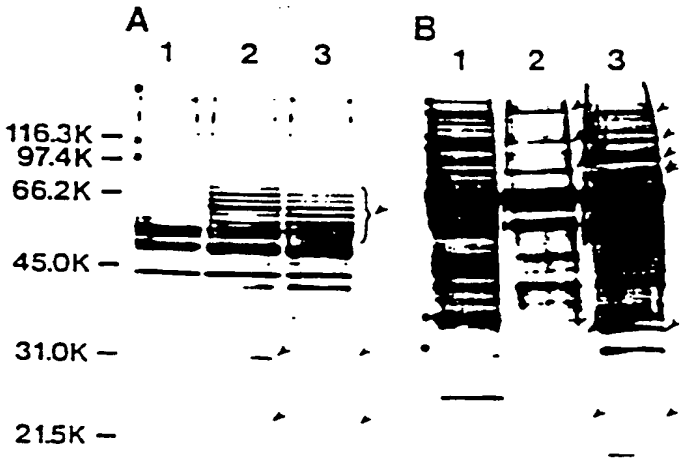


Fig 10

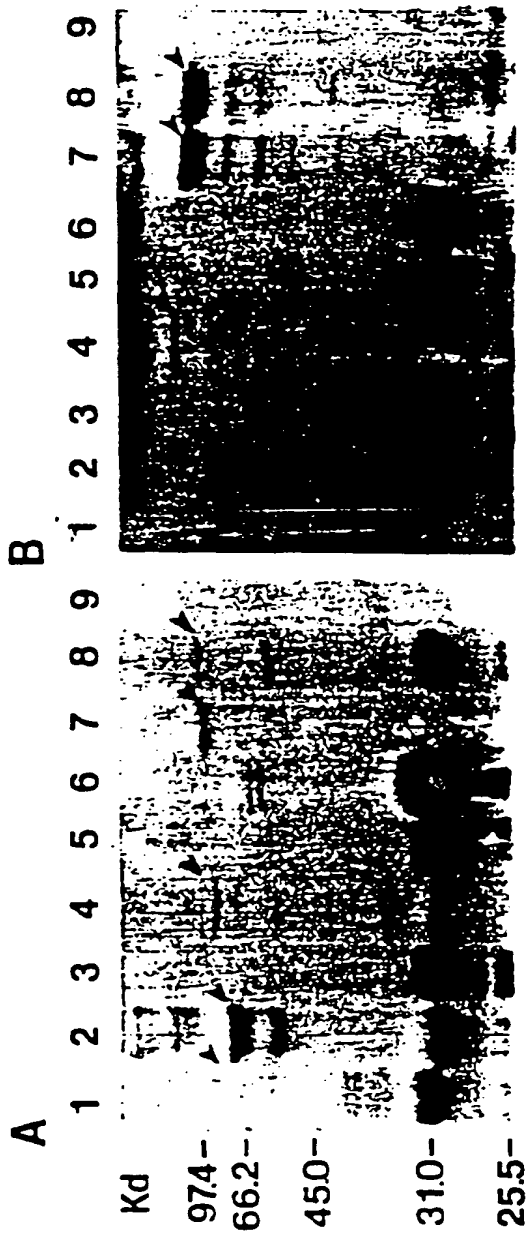
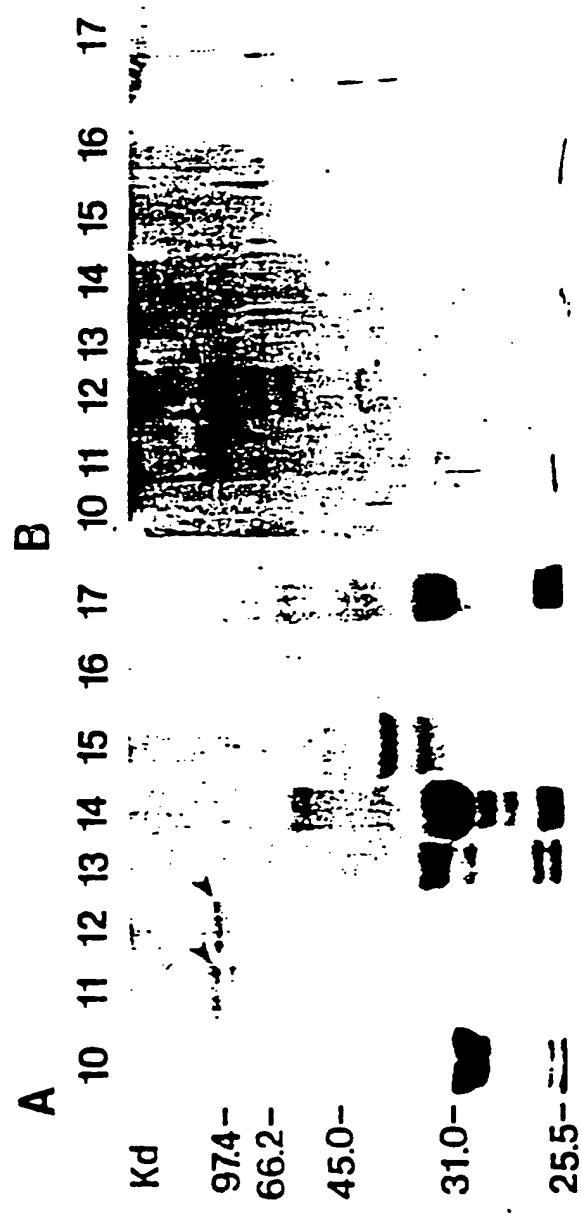


Fig. 11


A
10 11 12 13 14 15 16 17
Kd
97.4-
66.2-
45.0-
31.0-
25.5-
B
10 11 12 13 14 15 16 17

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 93 11 7929

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	EP-A-0 502 236 (BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH) 9 September 1992 * Whole document; specifically Fig. 3 *	1-15	C07K15/00 C12N15/34 A01N63/00
X,D	J. GENERAL VIROLOGY vol. 72, no. 11, November 1991, pages 2645 - 2651 YOSHIFUMI HASHIMOTO ET AL. 'Location and nucleotide sequence of teh gene encoding the viral enhancing factor of the Trichoplusia ni granulosis virus' * "Methods" and sequence of Fig. 2 *	1-15	
X	EP-A-0 384 294 (BOYCE THOMPSON INST. FOR PLANT RESEARCH) 29 August 1990 * Claims 1 to 15 and Fig. 3 *	1-15	
X	EP-A-0 336 341 (BOYCE THOMPSON INST. FOR PLANT RESEARCH) 11 October 1989 * Page 3, line1-16 and claims 1-9 *	1-3,9-11	TECHNICAL FIELDS SEARCHED (Int. Cl.5)
X,D	& US-A-4 973 667	1-3,9-11	C07K
A,D	J. INVERTEBRATE PATHOLOGY vol. 27, no. 1, January 1976, pages 115 - 124 S. HARA ET AL. 'Isolation and characterisation of synergistic enzyme ...'		

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	01 FEBRUARY 1994	Germinario C.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P0601)

P.B. 5818 - Patentlaan 2
2280 HV Rijswijk (ZH)
Tx 31651 epo
FAX (070) 340 3016

Europäisches Patentamt
Zweigstelle in Den Haag
Recherchenabteilung

European Patent Office
at Hague
Search Division

Office européen des brevets
Département à La Haye
Division de la recherche

Campbell, Patrick John Henry
J.A. Kemp & Co.,
14 South Square,
Gray's Inn
London WC1R 5JJ
GRANDE BRETAGNE

Datum/Date
11.03.03

Zeichen/Ref./Réf.	Anmeldung Nr./Application No./Demande n°.//Patent Nr./Patent No./Brevet n°
N.83606 PJC	00908824.6-2406/AU0000181

Anmelder/Applicant/Demandeur//Patentinhaber/Proprietor/Titulaire
COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION

COMMUNICATION

The European Patent Office herewith transmits the supplementary partial European search report under Rule 46(1) EPC relating to the above-mentioned European patent application.

Copies of the documents cited in the search report are enclosed.

The applicant's attention is drawn to the following:

The search Division informs the applicant that if the European search report is also to cover inventions other than the invention first mentioned in the claims, a further search fee must be paid for each of these inventions, within ONE MONTH after notification of this communication.

If the application has been filed up to 30 June 1999, the search fee in force before 01 July 1999 (EUR 869,--) or the equivalent applicable on the date of payment is payable.
This applies also to the search fees requested under Rule 46(1) EPC.
See also OJ EPO 06/1999, 405.

☐ The abstract was modified by the Search Division and the definitive text is attached to the present communication.

☒ Additional set(s) of copies of the documents cited in the European search report is (are) enclosed as well.

Note to users of the automatic debiting procedure:

Unless the EPO receives prior instructions to the contrary, the search fee(s) will be debited on the last day of the period for payment. For further details see the Arrangements for the automatic debiting procedure, Supplement to OJ EPO 02/1999.

REGISTERED LETTER

European Patent Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 00 90 8824

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1,2,7-10,15-21 partially; 3-6,11-14 completely

   Transformed plant comprising at least one polynucleotide molecule comprising a nucleotide sequence encoding a constituent protein from a spindle body or spindle -like body from an insect virus, said protein being fusolin or fusolin-like protein, and subject-matter related thereto.

   Method for controlling or preventing damage caused to plants from feeding insects, comprising applying to said plant a feed bait composition comprising fusolin or fusolin-like proteins, with the provisio that the feed bait composition does not further comprise a nuclear polyhedrosis virus, wherein said composition is applied before, after or together with an insecticidal and/or biological agent.

   Method for controlling or preventing damage caused to said transformed plants from feeding insects, comprising applying to said plant an insecticidal chemical and/or biological agent, with the provisio that said biological agent is not a polyhedrosis virus, and subject-matter related thereto.

2. Claims: 1,2,7-10,15-21 partially

   idem, wherein the constituent protein is an ER-specific chaperone BiP protein

European Patent Office

SUPPLEMENTARY PARTIAL EUROPEAN SEARCH REPORT

under Rule 46, paragraph 1 of th European Pat nt Convention

Application Number EP 00 90 8824

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	WO 97 12906 A (COMMW SCIENT IND RES ORG ;DALL DAVID JAMES (AU)) 10 April 1997 (1997-04-10) * the whole document *	1,7-9, 15-18,21	A01H5/00 A01N63/02
A	EP 0 596 508 A (THOMPSON BOYCE PLANT RES) 11 May 1994 (1994-05-11)		
P,X, D	HUKUHARA TOSIHIKO ET AL: "Increased baculovirus susceptibility of armyworm larvae feeding on transgenic rice plants expressing an entomopoxvirus gene." NATURE BIOTECHNOLOGY, vol. 17, no. 11, November 1999 (1999-11), pages 1122-1124, XP002231409 ISSN: 1087-0156 * the whole document *	1-8	

TECHNICAL FIELDS SEARCHED (Int.Cl.7)

A01N
C07K
C12N

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to severalinventions or groups of inventions, namely

see sheet B

The present partial European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

Place of search	Date of completion of the search	Examiner
MUNICH	17 February 2003	Kania, T

CATEGORY OF CITED DOCUMENTS

X particularly relevant if taken alone
Y particularly relevant if combined with another document of the same category
A technological background
O non-written disclosure
P intermediate document

T theory or principle underlying the invention
E earlier patent document, but published on, or after the filing date
D document cited in the application
L document cited for other reasons

& member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C23)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 00 90 8824

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

17-02-2003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9712906 A	10-04-1997	AU 7079296 A	28-04-1997
		WO 9712906 A1	10-04-1997
EP 0596508 A	11-05-1994	US 5475090 A	12-12-1995
		CA 2102387 A1	05-05-1994
		EP 0596508 A1	11-05-1994